

Lithium Ion Selective Optical Sensor Based on a Novel Neutral Ionophore and a Lipophilic Anionic Dye

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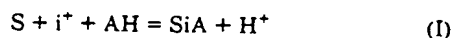
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INTRODUCTION

Lithium salts such as Li_2CO_3 have been used as a medicine for manic depressive and hyperthyroidism patients.¹ The Li^+ concentration in the serum of manic depressive patients was reported to be 0.5–1.5 mM, with over 1.5 mM being a toxic range.¹ Thus, a Li^+ sensor for monitoring Li^+ in blood fluids is desired in the medical and clinical fields in order to monitor and control the Li^+ concentration in the human body. For blood serum analysis, a high concentration of Na^+ coexists. Therefore, an important characteristic for the development of a Li^+ sensor is how to prevent or minimize any interference from Na^+ , which has an ionic diameter similar to Li^+ as well as having the same positive charge, being in the same periodical group of elements. The concentration of Na^+ in normal blood (extracellular) is about 140 mM and the concentration of Li^+ , which comes from the medicine, is at the millimolar level.^{1,2} Thus, an ion selectivity (Li^+/Na^+) of over 10 000 is needed for the selective Li^+ determination at the millimolar level.²

Unfortunately, any electrodes developed to date have never been able to maintain this required selectivity. Some electrodes have been reported to have a Li^+/Na^+ selectivity of over 100^{2-9} (over 1000;^{7,9} for a review, see ref 10). Therefore, the aim of this research was to develop an optical Li^+ sensor (Li^+ -selective optode) which is capable of determining low levels of Li^+ in the serum of manic depressive patients, which has never been realized using ion-selective electrodes.

In our recent research, a high-performance K^+ optical sensor was successfully developed using a highly selective neutral ionophore and a highly sensitive color-changeable anionic dye, in which the response mechanism is simply expressed by eq I based on the ion-pair formation reaction,¹¹ where S is a



neutral ionophore, i^+ is the ion to be sensed, AH is a lipophilic anionic dye, and SiA is a produced association (ion-pair) species. Based on this equation, these types of optodes can be created not only for K^+ but also for other specific sensors by replacing the ion-selective ionophore.

Here we report the first Li^+ -selective optode in which a similar response mechanism is represented by eq I. This optical Li^+ sensor can determine Li^+ by basically measuring

the change in absorbance (516 nm) of the newly synthesized color-changeable anionic dye of the diphenylamine type (LAD-3; see Figure 1). As the Li^+ ionophore, we designed and synthesized a 14-crown-4 derivative (PTM14C4; see Figure 1) having a bulky pinane and subunits at the ethano bridge of the crown ring, which prevents the formation of a 2:1 sandwich-type complex with large cations which do not fit the cavity size of the 14-crown-4. Consequently, a sensor having a high Li^+ selectivity of over 10 000 could be achieved against all alkali metal and alkaline earth metal cations except Li^+ . In addition, a flow-through-type optode was designed to realize practical continuous measurements of many aqueous samples with easy operation. The Li^+ optode was prepared by packing pellicular-type ODS beads (30–40 μm) coated with a newly synthesized lipophilic organic liquid (TFPDE; see Figure 1) incorporating the ionophore (PTM14C4) and the dye (LAD-3) in a flow-through optical cell (volume 7 μL) having a quartz window attached directly to the tip of a bifurcated optical fiber (see Figure 2), so that the fast color change due to the Li^+ concentration in the sample can be achieved. In this case, incident light of 516 nm was introduced through one of the bifurcated optical fibers and the resulting diffuse reflection light accompanying the absorbance change of the sensing beads was measured. This detection light mode was also effective for fast-response measurements.

Furthermore, the general response of these types of optodes was theoretically expressed with a formula, and the results were applied to the composition of the ion-sensing components to attain higher Li^+ sensitivity. As a result, a flow-through-type, highly Li^+ selective optode was developed and could be successfully applied to measure Li^+ -spiked serum samples adjusted to the therapeutic concentration range found in manic depressive patients.

THEORETICAL SECTION

Sensor Response. The response of the developed optical ion sensor based on the Li^+ -selective neutral ionophore and the lipophilic anionic dye (LAD-3) can be represented by expanding the ion-pair extraction system previously reported as an optical K^+ sensor using valinomycin and a lipophilic anionic dye, LAD,¹¹ which has a structure similar to that of LAD-3. The following equations can be adapted by any sensor systems based on a neutral ionophore and an anionic dye represented as eq I. For the case when an ion in aqueous solution forms a $p:m$ - (ion:ionophore-) type complex with a neutral ionophore, the sensor response can be generally expressed as eq II and demonstrated as shown in Figure 3



based on the ion-pair extraction reaction. Subscript o represents the organic phase, i is a cation to be sensed, S is a neutral ionophore, and AH is a lipophilic anionic dye of the protonated form. The measured chemical species on the optical ion sensor is the absorbance of $\text{S}_m\text{i}_p\text{A}_{n,o}$ in eq II. Using activities of chemical species in relation to the formation of this species ($a_{\text{S}_m\text{i}_p\text{A}_{n,o}}$), equilibrium constants can be represented as eqs 1–6, where a_i , a_{S_o} , a_{AH_o} , and a_{H^+} are activities of i, S_o ,

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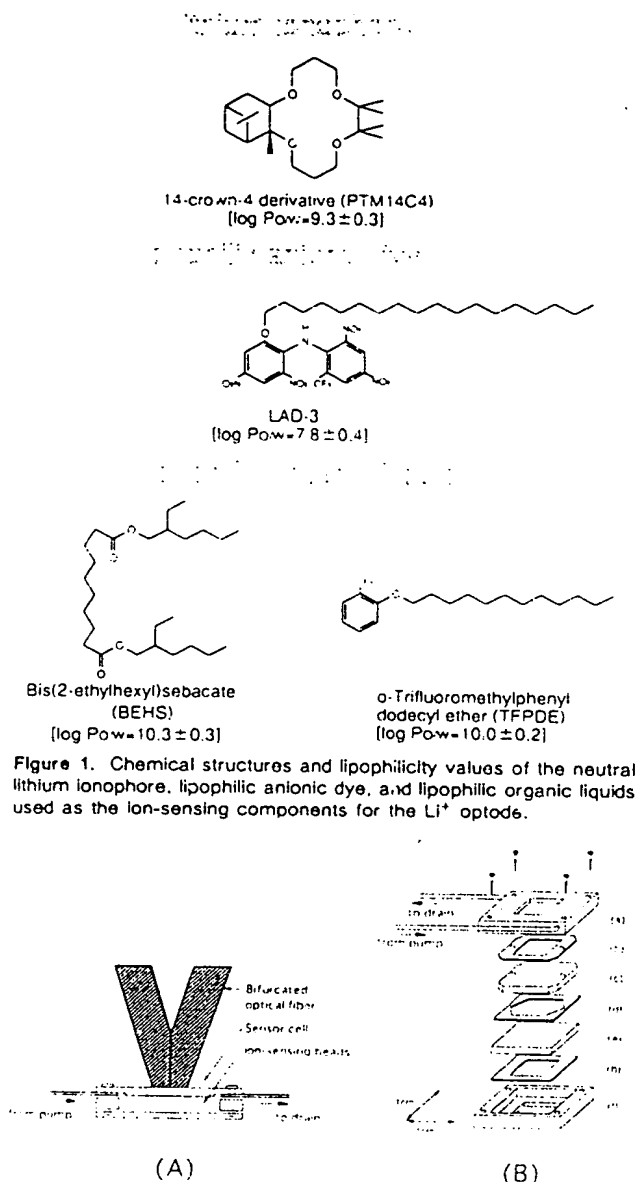


Figure 2. Schematic views of the flow-through-type optical sensor probe (A) and the optical sensor cell assembly (B): a. upper stainless steel cell body; b. silicon rubber packing; c. quartz glass window; d. Teflon spacer (0.05-mm thickness); e. mirror with a hydrophobic surface; f. lower stainless steel cell body.

$$k_S = a_{S_o} / a_S \quad (1)$$

$$k_{AH} = a_{AH_o} / a_{AH} \quad (2)$$

$$k_{S_{mip}A_n} = a_{S_{mip}A_{n,o}} / a_{S_{mip}A_n} \quad (3)$$

$$\beta_{Si} = a_{S_{mip}A_n} / a_S^m a_{i^{n+}}^p \quad (4)$$

$$K_a = (a_{A^-} - a_{H^+}) / a_{AH} \quad (5)$$

$$K_{AA} = a_{S_{mip}A_n} / a_{S_{mip}A_n}^m a_{A^-}^n \quad (6)$$

AH_o , and H^+ , respectively, β_{Si} is the stability constant of $S_{mip}A_n^{p+}$, K_{AA} is the association constant of $S_{mip}A_n^{p+}$ and A^- , K_a is the dissociation constant of the anionic dye, AH , and k_S , k_{AH} , and $k_{S_{mip}A_n}$ are the partition constants of S , AH , and

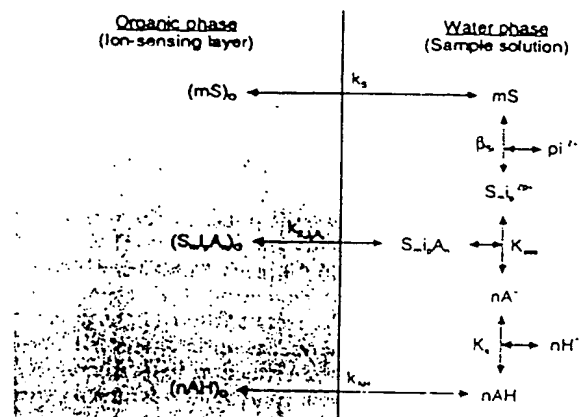


Figure 3. Ion extraction system for explaining the optical cation sensor response: S , neutral ionophore; AH , anionic dye of the protonated form; i^{n+} , ion to be sensed (n , charge number). The subscript o represents the organic phase. For factors and coefficients, see Results and Discussion.

$S_{mip}A_n$, respectively, in the distribution equilibrium between the organic phase (lipophilic sensing phase) and the aqueous phase (sample solution). Hence, the activity of the species contributing to the absorbance change, $a_{S_{mip}A_{n,o}}$, can be expressed as eq 7 using eq 1-6. This equation is further

$$a_{S_{mip}A_{n,o}} = \frac{k_{S_{mip}A_n} K_{AA} \beta_{Si} K_a^n a_{i^{n+}}^p a_{S_o}^m a_{AH_o}^n}{k_S^m k_{AH}^n a_{H^+}^n} \quad (7)$$

modified as eq 12 with eq 7 representing the summarized constant, K , and eqs 9-11 relating the mass and charge balances of the chemical species.

$$K = \frac{k_{S_{mip}A_n} K_{AA} \beta_{Si} K_a^n}{k_S^m k_{AH}^n} \quad (8)$$

$$a_{i^{n+}}^{tot} = a_{S_o} + m a_{S_{mip}A_{n,o}} = q_S C_S^{tot} \quad (9)$$

$$a_{AH}^{tot} = a_{AH_o} + m a_{S_{mip}A_{n,o}} = q_{AH} C_{AH}^{tot} \quad (10)$$

$$zp = n \quad (\text{for the electroneutrality}) \quad (11)$$

$$a_{S_{mip}A_{n,o}} = \frac{K a_{i^{n+}}^p (a_S^{tot} - m a_{S_{mip}A_{n,o}})^m (a_{AH}^{tot} - n a_{S_{mip}A_{n,o}})^n}{a_{H^+}^n} \quad (12)$$

K represents the ion-pair extraction equilibrium constant, a_{AH}^{tot} and a_S^{tot} are the total anionic dye and the total neutral ionophore activities, C_S^{tot} and C_{AH}^{tot} are the total concentrations of the neutral ionophore and of the anionic dye, and q_S and q_{AH} are the activity factors of S and AH in the organic phase. The values of the activity factors are assumed to be nearly constant ($q_S = q_{AH} = 1$) and not dependent on the concentrations of S and AH in the organic phase. The response of the sensor, in which the light was introduced through one branch of the bifurcated optical fiber and the transmittance light was detected through the ion-sensing phase from the other fiber, can be expressed as eq 14, based on the Lambert-Beer equation indicated as eq 13.¹¹ A represents the absorbance detected, ϵ is the molar absorption coefficient of the dye ($S_{mip}A_{n,o}$ form), and b is the effective

$$A = \Phi \epsilon b a_{S_{i,p}A_n} \quad (13)$$

$$a_{i,p} = \frac{a_{H^+}^n A (\Phi \epsilon b)^{m+n-1}}{K (\Phi \epsilon b a_S^{\text{tot}} - m A)^m (\Phi \epsilon b a_{AH}^{\text{tot}} - n A)^n} \quad (14)$$

$$(\Phi \epsilon b a_S^{\text{tot}} \geq m A \geq 0, \Phi \epsilon b a_{AH}^{\text{tot}} \geq n A \geq 0)$$

light path length of the organic phase as the sensing layer. The factor Φ is the detection light collection efficiency. The combination of eqs 12 and 13 leads to eq 14, which is the fundamental equation for expressing the response of the optical sensor based on the transmittance light mode measurement. On the other hand, the optical sensor is based on the measurement of diffuse reflection light; therefore, the sensor response is expressed by Kubelka-Munk's equation (eq 15) instead of the Lambert-Beer equation (eq 13).¹³ Thus,

$$f(r) = \frac{(1 - 10^{-A})^2}{2 \times 10^{-A}} = \frac{a_{S_{i,p}A_n}}{\Psi} \quad (15)$$

$$a_{i,p} = \frac{a_{H^+}^n \Psi f(r)}{K (a_S^{\text{tot}} - m \Psi f(r))^m (a_{AH}^{\text{tot}} - n \Psi f(r))^n} \quad (16)$$

$$(a_S^{\text{tot}} > m \Psi f(r) \geq 0, a_{AH}^{\text{tot}} > n \Psi f(r) \geq 0)$$

the response of the sensor investigated herein (see Figure 2) is represented as eq 16, where $f(r)$ is the Kubelka-Munk factor measured as the sensor response in the absorbance mode and Ψ represents the instrument factor with consideration of straying and scattering of the incident light, the thickness of the ion-sensing layer, and the density of the particle packed in the cell. For both types of sensors based on the measurement of transmittance light or diffuse reflection light, the optical response is determined by six important factors: (1) the value of the constants (K) relating ion-pair extraction equilibrium of the species of $S_{i,p}A_n$, (2) the sample solution pH (proton activity a_{H^+}), (3) the activity of the total anionic dye (a_{AH}^{tot}), (4) the mixing ratio of the neutral ionophore and the anionic dye ($a_S^{\text{tot}}/a_{AH}^{\text{tot}}$), (5) the stoichiometry (1: m) of the complex formed with the neutral ionophore, S , and the cation, i (in the case, the important species concerning the optical detection is $S_{i,p}A_n$ formed with the cationic complex species, $S_{i,p}^{p+}$ and anionic dye, A , where $S_{i,p}A_n$ is electrically neutral in the bulk optode sensing phase and the value of n is the same as the number of the charge, z^{p+}), and (6) the charge number (z) of the ion, i , to be sensed.

Sensor Selectivity. Based on the theoretical response as expressed with eq 16, the ion selectivity coefficient ($k_{i,j}^{\text{opt}}$, where i is the primary ion and j is the interfering ion) of the optical sensor is basically defined as eq 17, which can be

$$A = A_i + k_{i,j}^{\text{opt}} A_j \quad (17)$$

$$k_{i,j}^{\text{opt}} = a_i'/a_j \quad (= a_i'/a_j) \quad (18)$$

determined graphically in the same manner as the separate solution method SSM for an ion-selective electrode.¹⁴ In this case, if the interfering ion (j) has the same charge as that of the primary ion (i), their response curves have to be drawn as shown in Figure 4a, and the ion-selectivity coefficient of the optical ion sensor ($k_{i,j}^{\text{opt}}$) can simply be calculated with eq

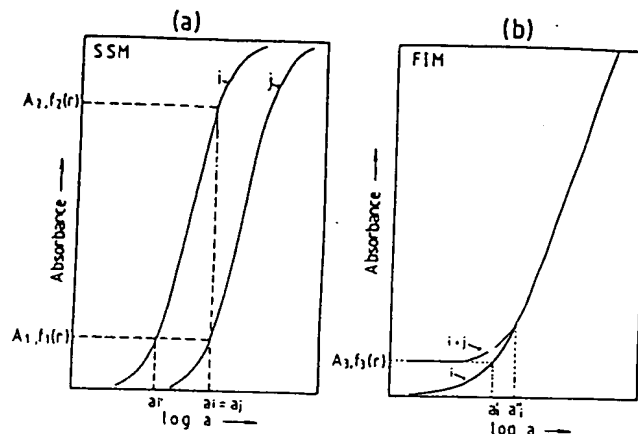


Figure 4. Typical relationship between the absorbance [A , or the Kubelka-Munk factor, $f(r)$] as the sensor response and measured ion activity (a_i or a_j) for understanding the selectivity coefficient, $k_{i,j}^{\text{opt}}$ (i , primary ion; j , interfering ion), determined by (a) the separate solution method (SSM) or (b) the fixed interference method (FIM).

18, where A_i and A_j are the measured absorbances for cations i and j , respectively, at fixed ion concentrations ($a_i = a_j$). A_1 and A_2 in Figure 4a are the absorbances corresponding to $f_1(r)$ and $f_2(r)$ for the optical sensor based on the diffuse reflection light mode measurement, and the corresponding activities, a_i ($=a_j$) and a_i' , are determined by the adjunct method with dashed lines described over the calibration graphs for the responding cations i and j in Figure 4a.

In general, when the primary (i) and interfering ion (j) form 1: m_1 - and 1: m_2 -type (ion:ionophore) complexes, respectively, where a neutral ionophore and the resulting charge numbers of these complexes are n_1 and n_2 , respectively, eq 18 is further expressed using eq 16 as eq 19a or 19b. This (eq

$$k_{i,j}^{\text{opt}} = \frac{f_1(r) (a_S^{\text{tot}} - m_1 \Psi f_2(r))^{m_1} (a_{AH}^{\text{tot}} - n_1 \Psi f_2(r))^{n_1}}{f_2(r) (a_S^{\text{tot}} - m_1 \Psi f_1(r))^{m_1} (a_{AH}^{\text{tot}} - n_1 \Psi f_1(r))^{n_1}} \quad (19a)$$

$$\left(= \frac{a_{H^+}^{n_1} K_j (a_S^{\text{tot}} - m_2 \Psi f_1(r))^{m_2} (a_{AH}^{\text{tot}} - n_2 \Psi f_1(r))^{n_2}}{a_{H^+}^{n_1} K_i (a_S^{\text{tot}} - m_1 \Psi f_1(r))^{m_1} (a_{AH}^{\text{tot}} - n_1 \Psi f_1(r))^{n_1}} \right) \quad (19b)$$

19a or 19b) is the basic equation calculated from the measured absorbances of the primary ion [A_i or $f_1(r)$] and interfering ion [A_j or $f_2(r)$]. When the measured ion concentration was fixed, the selectivity coefficient of the optical ion sensor could be determined using eq 19a or 19b, and the determined value itself is meaningful and comparable. However, there is a problem for the case where the selectivity factors vary with activities of the primary as well as the interfering ion, because they exhibit different response curve shapes. For a more reliable way to determine the selectivity coefficient, we recommend the graphical method shown in Figure 4b, which is similar to the fixed interference method (FIM) for an ion-selective electrode.¹⁴ In this case, the two sensor response curves obtained with an aqueous solution containing only the primary ion (i) and with an aqueous solution containing the primary ion as well as the interfering ion ($i + j$), are used to determine the selectivity factor. The latter response curve exhibits a poorer response than the former one in the low-activity range because of the interference from the coexisting interfering ion. In this case, the selectivity coefficients can be determined using eq 20, where a_i' is an activity which

$$k_{i,j}^{\text{opt}} = a_i'/a_j \quad (20)$$

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exhibits the same absorbance (A_3 or $f_3(r)$ in Figure 4b) in the primary ion response (i) curve when the coexisting interfering ion (j) is fixed and affected by the response for i . In this case, the effective calibration activity range for the optical ion sensor is also determined, which is over the lowest activity, a_i^{**} (determination limit; see Figure 4b), in the calibration curve for the primary ion, in which the absorbance obtained with the two response curves are quite identical.

EXPERIMENTAL SECTION

Reagents. Reagents of the highest grade commercially available were used for syntheses of the new compounds and preparation of the aqueous test electrolytes. Distilled, deionized water had resistivity values of more than $1.5 \times 10^7 \Omega \text{ cm}$ at 25 °C. Pericurar-type ODS bead was purchased from Merck (Perisorb RP-18; 30–40- μm particle size, approximately 1–2- μm porous surface region which was ODS-modified, approximately 14 m^2/g , 0.05 mL/g of pore volume). Bis(2-ethylhexyl) sebacate (BEHS; see Figure 1 for the structure; purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) and a newly synthesized *o*-(trifluoromethyl)phenyl dodecyl ether (TFPDE) were used as the lipophilic organic liquids. The serum samples were supplied from Ortho Diagnostics Inc., Raritan, NJ.

Synthesis of 14-Crown-4 Derivative. The novel Li^+ ionophore was synthesized according to the following procedures. Pinacol (Tokyo Chemical Industry), 6.15 mmol (0.73 g), and sodium hydride, 18.45 mmol (0.44 g), were added in 10 mL of absolute tetrahydrofuran (THF) and stirred at ambient temperature for 30 min. Then 12.30 mmol (1.49 g) of allyl bromide dissolved in 3 mL of THF was further added in the reaction mixture and stirred at 80 °C for 36 h. After the addition of small amounts of methanol to the reaction mixture, the THF was evaporated. The resulting residue was extracted three times with ethyl acetate. After the organic phase was dried with Na_2SO_4 and evaporation, the obtained residue was purified by silica gel column chromatography with hexane–ethyl acetate (4:1) as the eluent to yield 4,7-dioxo-5,5,6,6-tetramethyl-1,9-decane (0.57 g, 46.7%). This product, 2.88 mmol (0.57 g), NaBH_4 , 2.59 mmol (0.10 g), and boron trifluoride ethyl ether complex, 3.44 mmol (0.49 g), were added to 3 mL of THF and stirred at ambient temperature for 2 h. After the addition of small amounts of deionized water, 2.1 mmol (0.08 g) of NaOH and 30% H_2O_2 aqueous solution (0.90 mL) were added to the reaction mixture and the resultant mixture was stirred for 2 h. The product, 4,7-dioxo-5,5,6,6-tetramethyldecane-1,10-diol (compound a), was extracted from the reaction mixture with ethyl acetate and purified by silica gel column chromatography with hexane–ethyl acetate (1:1) as the eluent (yield: 0.28 g, 41.5%). 4,7-Dioxo-5,5,6,6-tetramethyldecane-1,10-diol bis(*p*-toluenesulfonate) (compound b) was prepared from compound a and *p*-toluenesulfonyl chloride in absolute pyridine (yield: 0.5 g, 54.2%). (1*R*,2*R*,3*S*,5*R*)-(-)-Pinanediol (Aldrich Chemical Co., Inc., Milwaukee, WI) (0.92 mmol) and 2.76 mmol of NaH were added to 10 mL of THF and the resultant mixture was refluxed for 30 min. Compound b (0.92 mmol) dissolved in 10 mL THF was added to the reaction mixture and the mixture was refluxed for 60 h. After small amounts of methanol were added, the reaction mixture was evaporated to dryness. The obtained residue was extracted with chloroform, and the organic phase was dried with Na_2SO_4 . Finally, after evaporation of the organic phase, the primary product, 2,3-((1*R*)-(1*a*,2*a*,3*a*,5*a*))-2,6,6-trimethylbicyclo-[3.1.1]heptano)-9,9,10,10-tetramethyl-1,4,8,11-tetraoxacyclotetradecane (PTM14C4; see Figure 1) was purified by silica gel column chromatography with hexane–ethyl acetate (20:1) as the eluent (yield: 87.0 mg, 25.6%). This final product was further purified using reversed-phase HPLC (column, ODS; eluent, methanol).

The analytical data for PTM14C4 obtained with ^1H NMR (270 MHz, CDCl_3), IR (KBr), and elemental analyses were as follows: mp 67.5–68.5 °C; ^1H NMR, δ 0.92 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 1.18 (s, 3H, CH_3), 1.20 (s, 3H, CH_3), 1.22 (s, 3H, CH_3), 1.25 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.52 (d, 1H, CH), 1.60–1.90 (m, 6H, CH_2), 1.98–2.10 (m, 2H, CH_2), 2.26–2.38 (m, 1H, CH), 3.30–4.10 (m, 9H, OCH_2 , OCH); IR (KBr), 1107, 2850, 2917 cm^{-1} .

Anal. Calcd for $\text{C}_{22}\text{H}_{40}\text{O}_4$ (368.56): C, 71.70; H, 10.94. Found: C, 71.60; H, 10.78.

Synthesis of a Lipophilic Anionic Dye (LAD-3). LAD-3 was synthesized according to the following six-step reaction with *o*-nitrophenol as the starting material. *o*-Nitrophenol (3 g) and NaH (1.28 g) were added to 30 mL of absolute *N,N*-dimethylformamide (DMF) and stirred for 1 h at 0 °C. Stearyl bromide, 7.1 g, was added dropwise to this reaction mixture and the resultant mixture was refluxed for 24 h. After the addition of 2 mL of methanol to the reaction mixture and concentration, the product, *o*-nitrophenyloctadecylether (*o*-NPODE), was extracted from the reaction mixture with ethyl acetate and purified by silica gel chromatography with hexane–ethyl acetate (2:1) as the eluent (60% yield).

o-NPODE, 3 g, was dissolved in 20 mL of hexane and catalytically reduced to 2-octadecyloxylaniline (ODA) with 400 mg of 10% palladium carbon powder in an autoclave under 30 atm hydrogen atmosphere at ambient temperature for 24 h (100% yield). The following methods for obtaining LAD-3 with ODA were similar to the reported procedures for the synthesis of LAD.¹¹ The final product, *N*-2,4-dinitro-6-(octadecyloxy)phenyl-2',4'-dinitro-6'-(trifluoromethyl)phenylamine (LAD-3; see Figure 1), was extracted with chloroform and purified by silica gel chromatography with hexane–ethyl acetate (1:6) as the eluent (60% yield).

The analytical data for LAD-3 obtained with ^1H NMR (270 MHz, CDCl_3), IR (KBr), and elemental analyses were as follows: yellow needles; mp 90.5–92.0 °C; ^1H NMR, δ 0.88 (t, 3H, CH_3), 1.2–1.5 (br, 30H, CH_2), 1.84 (m, 2H, CH_2), 4.22 (t, 2H, OCH_2), 7.11 (s, 1H, aromatic), 7.38 (s, 1H, aromatic), 7.58 (s, 1H, NH), 8.22 (s, 1H, aromatic), 9.04 (s, 1H, aromatic); IR (KBr), 1346, 1528, 1552, 1625, 2362, 2852, 2917, 3430 cm^{-1} . Anal. Calcd for $\text{C}_{31}\text{H}_{41}\text{N}_5\text{O}_9\text{F}_3$ (685.70): C, 54.46; H, 6.04; N, 10.09. Found: C, 54.30; H, 6.17; N, 10.21.

Synthesis of *o*-(Trifluoromethyl)phenyl Dodecyl Ether (TFPDE). Sodium hydride, 178 mg (7.4 mmol), and dodecyl bromide, 1.54 g (6.17 mmol), were added to a solution of 1.0 g (6.17 mmol) of *o*-(trifluoromethyl)phenol (Aldrich) in 15 mL of dimethylformamide and stirred at 70 °C for 3 h. The reaction mixture was evaporated, and the obtained residue was further extracted three times with chloroform. The organic phase was dried with anhydrous sodium sulfate and evaporated. Finally, the product, 1.7 g (5.14 mmol, viscous colorless oil, 83.5%) of TFPDE (see Figure 1), was purified by silica gel chromatography with hexane as the eluent.

The constitution of the synthesized lipophilic organic liquid was confirmed by ^1H NMR (270 MHz, CDCl_3), IR (CHCl_3), and elemental analysis. For TFPDE: $\nu_{\text{C-F}}$, 1118, 1136, $\nu_{\text{C-O}}$, 1461, 1497, 1591, 1610, ν_{CH} , 2855, 2928 cm^{-1} ; ^1H NMR, δ 0.88 (t, 3H, CH_3), 1.25–1.40 (m, 16H, CH_2), 1.40–1.55 (m, 2H, CH_2), 1.75–1.90 (m, 2H, CH_2), 4.05 (t, 2H, OCH_2), 6.9–7.0 (m, 2H, aromatic), 7.40–7.50 (t, 1H, aromatic), 7.50–7.60 (d, 1H, aromatic). Anal. Calcd for $\text{C}_{19}\text{H}_{35}\text{OF}_3$ (330.43): C, 69.15; H, 8.98. Found: C, 69.06; H, 8.85.

Preparation of Lithium Ion Sensing Beads. The Li^+ -sensing organic liquid was prepared by dissolving PTM14C4 as the neutral lithium ionophore and LAD-3 in the lipophilic organic liquid of BEHS or TFPDE using a 10-mL glass vessel. The chemical structures of these organic liquid components are shown in Figure 1. The Li^+ -sensing ODS beads were prepared by mixing pellicular ODS beads with the Li^+ -sensing liquid having the following compositions in a glass vessel. Five kinds of Li^+ -sensing beads were prepared: 100 mg of ODS beads, 40 mg of BEHS, 2.8 μmol of PTM14C4, and 5.6 μmol of LAD-3 as composition a; 200 mg of ODS beads, 80 mg of BEHS, 2.80 μmol of PTM14C4, and 5.6 μmol of LAD-3 as composition b; 200 mg of ODS beads, 80 mg of BEHS, 5.60 μmol of PTM14C4, and 5.6 μmol of LAD-3 as composition c; 200 mg of ODS beads, 80 mg of BEHS, 5.6 μmol of PTM14C4, and 2.8 μmol of LAD-3 as composition d; and 200 mg of ODS beads, 80 mg of TFPDE, 5.6 μmol of PTM14C4, and 5.6 μmol of LAD-3 as composition e.

Structure of the Flow-Through-Type Fiber-Optic Lithium Ion Sensor. The schematic structure of the fiber-optic Li^+ sensor probe is shown in Figure 2A. The optical measurement system was almost the same as the optical K^+ sensor reported previously which was constructed with a light source, the

bifurcated optical fibers, a light detector, and a flow-through cell sensor probe (different from the previous batchwise probe).¹¹ The diffuse reflection light on the surface of the Li⁺-sensing beads, which corresponds to the response of the sensor, was measured with a photodiode detector through a monochromator attached to a double-beam spectrophotometer (U-2000; Hitachi Co., Ltd., Tokyo, Japan). The structures of the sensor cell assemblies are shown in Figure 2B. The Li⁺-sensing beads were placed on the mirror as shown in Figure 2e and packed in a flow-through optical cell (volume 7 μ L) having a quartz window (Figure 2c) attached directly to the tip of a bifurcated optical fiber. The flow-through cell was set in the shield box. A mirror as shown in Figure 2e had a hydrophobic surface modified with triethoxyoctadecylsilane in order to prevent the beads from flowing out of the cell. The reference flow-through cell had the same structures as that of the sample cell. The same beads but not containing LAD-3 were packed in the reference cell, which was filled with pH-adjusted 0.05 M Tris-HCl buffer solution and attached in the same way as that for the Li⁺ sensor fiber. The light from the same source as that of the Li⁺ sensor fiber was introduced into the reference detector of the double-beam spectrophotometer via a bifurcated fiber. As the light source, a 150-W xenon lamp equipped with a spectrofluorometer (FP-550; Jasco, Tokyo, Japan) was utilized.

For the flow-through system, pH-adjusted 0.05 M Tris-HCl buffer solution was pumped at a flow rate of 1.0 mL/min using a pulse-free liquid delivery pump (Trirotar; Jasco) attached to the flow system. All sample solutions were also prepared with pH-adjusted 0.05 M Tris-HCl buffer solution and introduced from an HPLC injector (Rheodyne, Type 7025) having a 2-mL injection loop. The flowing and sample solution pH were adjusted similarly in the range from 6.0 to 8.0 according to the respective investigation.

Determination of Lipophilicities for Ion-Sensing Components. The lipophilicities, $\log P_{ow}$ (distribution coefficient between organic liquid and water) of the synthesized compounds (a neutral lithium ionophore, a lipophilic anionic dye, and lipophilic organic liquids) were calculated from the R_f values of reversed-phase thin-layer chromatography (RP-TLC) according to the method reported by Simon et al.¹² The RP-TLC plates (KC18F, Whatman) were 180 \times 200 mm and were chromatographically developed with ethanol-water (90:10) as the mobile phase.

RESULTS AND DISCUSSION

Sensor Characteristics. For Li⁺-selective ionophores, some derivatives of the 14-crown-4 have been reported to have a very high Li⁺ selectivity of over 1000 against Na⁺,^{7,9} which is a serious interference when the sensor is applied to measure Li⁺ in biological samples such as serum. We recently reported that introduction of a bulky subunit on the ethano bridge of a 14-crown-4 skeleton is very effective to attain high Li⁺/Na⁺ selectivity.^{9,15} Among all the 14-crown-4 derivatives, a decalino-14-crown-4 has the highest Li⁺ selectivity against Na⁺ ($k_{LiNa}^{pot} = 2000$).⁹ Similar excellent Li⁺ selectivity was observed for the pinane-14-crown-4 derivative, which has a pinane subunit on the ethano bridge of the cyclic skeleton.¹⁵ The detection limit was 1×10^{-6} M Li⁺ on the optimal Li⁺ optodes for both these two 14-crown-4 derivatives. However, at least a 5 times lower detection limit (2×10^{-6} M Li⁺) is desired for the accurate determination of Li⁺ (0.2–2 mM Li⁺) in the serum sample of manic depressive patients. As shown in Figures 6–8, the Li⁺ optode using a PTM 14-crown-4 as the ion-sensing Li⁺ ligand meets this required sensitivity. This indicates that a PTM 14-crown-4 has a high K value in eq 16, where the ion-ionophore complex stability constant (β_{Si}) or ion-ionophore-anionic dye complex (β_{SiA}) stability is larger than those with a decalino-14-crown-4 or pinane 14-crown-4 derivative as the Li⁺ ionophore. S. Judging from their structures, the tetramethyl ethano subunit is effective for

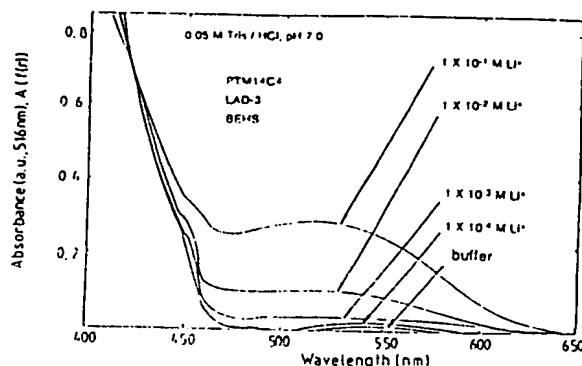


Figure 5. Absorption spectra obtained with the flow-through-type Li⁺ optode when different concentrations of Li⁺ (LiCl) were introduced. All samples and flowing solutions contain 0.05 M Tris-HCl, pH 7.

increasing the stability of the Li⁺ complex. As for Li⁺ selectivity, the optical sensor based on the 14-crown-4 derivative (PTM14C4), LAD-3, and BEHS exhibits absolutely no response to all alkali metal and alkaline earth metal cations up to 10^{-1} M except for Li⁺. Thus, the selectivity coefficients of the sensor are at least less than $1:10\,000$ ($\log k_{Li}^{pot} \leq -4$; j , interfering ion).

On the other hand, the highly sensitive color-changeable dye synthesized and used was of the diphenylamine type, which deprotonates at neutral pH [$pK_a = 6.91$, in dioxane-0.05 M Tris-HCl (1:1); molar extinction coefficient 2.13×10^4 L mol⁻¹ cm⁻¹ at 516 nm]. This anionic dye, LAD-3, has a structure basically similar (see Figure 1) to the LAD dye which was previously reported and used for the optical K⁺ sensor.¹¹ The newly synthesized dye (LAD-3) has an octadecyl side chain that improves lipophilicity and realized a $\log P_{ow}$ value over 10⁷, so that sensor life was improved. The other favorable feature of the anionic dye is that it does not form a precipitating complex with any cations, so that the dye maintains homogeneity in the lipophilic organic liquid on the ODS bead as the ion-sensing component (we have synthesized many anionic dyes and chromoionophores, but most of them often form an insoluble complex with some cations, so that they can hardly be used as the optical sensor component).

Figure 5 shows the typical absorption spectra of the sensing beads based on PTM14C4 and LAD-3. These highly sensitive ion-sensing beads turn from pale yellow to red when they are put in a sample solution containing Li⁺. As shown in the absorption curves of Figure 5, 480–550 nm is the most effective wavelength range for the sensing beads to detect Li⁺. The absorbance maximum, ϵ_{max} , of the sensing beads is 516 nm, which is the same as the ϵ_{max} for LAD-3. As we expected, the time for the color change of the sensing beads themselves was very fast (within a few seconds), when the beads are separately dispersed. The base bead (Perisorb RP-18, Merck) is a special type. It is a pellicular-type ODS bead covered a polar material of about 1–2- μ m thickness on the surface of a 30–40- μ m hard core particle. The sensing organic liquid was thinly coated on the surface of the beads. Thus, a fast response was attained. PVC was often used as the sensing membrane material, which was also used in our former optical sensors.^{11,16,19} In general, a PVC sensing membrane is highly lipophilic and the response

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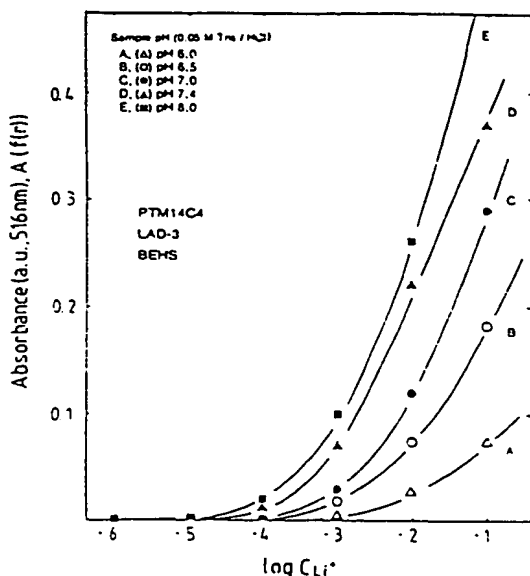


Figure 6. Typical response curves for Li^+ obtained with the flow-through-type Li^+ optode when the pH of the flowing solution was varied. All samples and flowing solutions contain 0.05 M Tris-HCl.

is not fast. In addition, a PVC membrane is gradually whitened and causes the sensitivity to decrease. The response time, of course, depends on the thickness of the sensing membrane, another negative factor that causes insensitivity, because it depends on the dye concentration and the membrane thickness. For these reasons, PVC was not used as the sensing membrane material.

Typical Li^+ response curves are shown in Figure 6, where the sample pH was at five different values in the range from pH 6.0 to 8.0. As explained with eq 16, the sensor sensitivity increases with increasing pH of the sample. Though the higher pH is effective for Li^+ detection sensitivity, a longer return time was needed for the color change for the Li^+ -free blank buffer solution, which was used after the injection of a high-concentration Li^+ sample of over 10^{-2} M. Therefore, pH 7.0 or 7.4 is the optimal sample pH condition for the Li^+ optode. A Tris buffer salt concentration of up to 0.2 M in the sample and flowing solution did not affect the color change of the sensing beads. As explained previously, the sensor sensitivity is expected to be affected by the contents of the Li^+ -selective ionophore (PTM14C4) and the anionic dye (LAD-3). When the ionophore/dye ratio of 1:1 (0.035/0.035 in mol/L) was used for the sensing components, the sensor response was curve c (this curve is the same as curve C in Figure 6) in Figure 7, where the detection limit is 1×10^{-4} M Li^+ . As expected from eq 16, varying the ratio of ionophore/dye increases sensitivity, and the resulting response curves b (ionophore/dye ratio of 1:2, 0.035/0.070 in mol/L) in Figure 7 had obviously increased sensor sensitivity. The highest sensitivity was observed in the sensor in the case where the sensing component of ionophore/dye was 0.067/0.134 (1:2; in mol/L) in which the anionic dye (LAD-3) as well as the ionophore contents were almost saturated.

One of other components which affects sensor sensitivity is the species of the lipophilic organic liquid used for dissolving the neutral ionophore and the anionic dye. All sensors used to obtain the response curves in Figures 5–7 utilized bis(2-ethylhexyl) sebacate as the organic liquid which was thinly coated on the ODS beads. The response curves in Figure 8 was obtained with the sensor, where the coating liquid of BEHS having a low polarity ($\epsilon = 4$; ϵ , dielectric constant) was replaced with *o*-(trifluoromethyl)phenyl dodecyl ether having

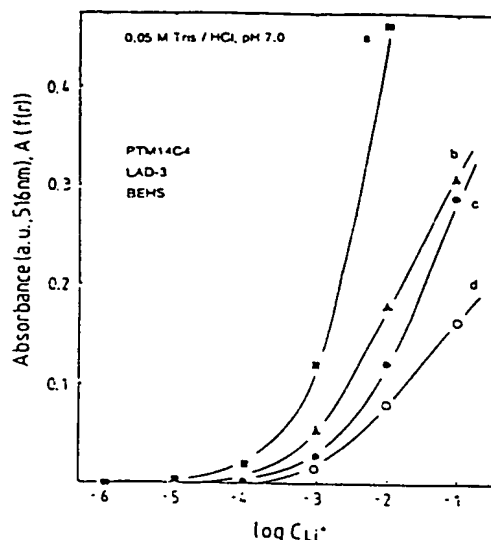


Figure 7. Typical response curves for Li^+ obtained with the flow-through-type Li^+ optode when the contents of the ion-sensing components (PTM14C4 and LAD-3) were varied. Mixing ratios of the ionophore (PTM14C4)/anionic dye (LAD-3) (in mol/L): a, 0.067/0.134; b, 0.035/0.070; c, 0.035/0.035; d, 0.070/0.035. All samples and flowing solutions contain 0.05 M Tris-HCl, pH 7.

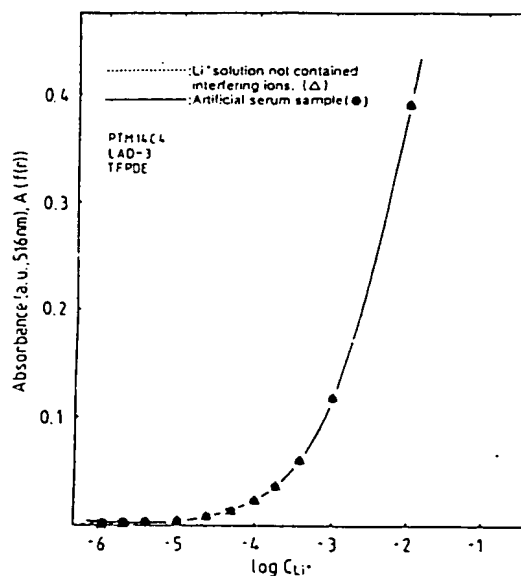


Figure 8. Typical response and calibration curves for Li^+ obtained with the flow-through-type Li^+ optode based on the Li^+ ionophore (PTM14C4), anionic dye (LAD-3), and lipophilic organic liquid (TFPDE). Mixing ratio of the ion-sensing components, PTM14C4/LAD-3 (in mol/L), 0.035/0.035. Test samples contain 15 mM Na^+ , 0.5 mM K^+ , 0.12 mM Ca^{2+} , and 0.08 mM Mg^{2+} (1:10 dilution of typical artificial serum samples). All sample and flowing solutions contain 0.05 M Tris-HCl, pH 7.

a higher polarity compared with that of BEHS. The higher polarity liquid is favorable in sensitivity because it brings a large extraction constant (K) for the ion-pair complex as the sensing chemical species (see eq 16). The new lipophilic organic liquid, TFPDE, was designed and synthesized on the basis of 2-nitrophenyl octyl ether (NPOE), which was often used as the membrane solvent for ion-selective electrodes of the poly(vinyl chloride) matrix membrane type. The nitro group produces the yellow color. Therefore, the polar group was replaced with a trifluoromethyl group to obtain novel colorless liquid, TFPDE (see Figure 1). In addition, the alkyl

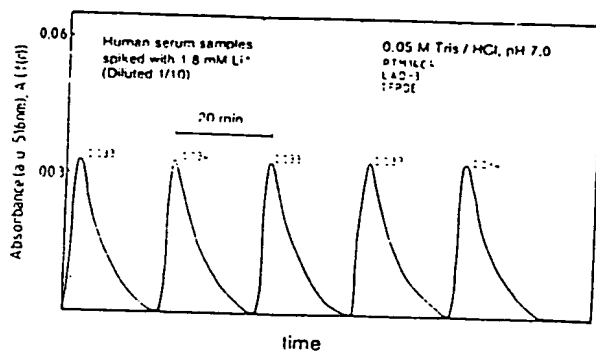


Figure 9. Typical response profiles for the human serum samples including Li^+ obtained with the flow-through-type Li^+ optode based on the Li^+ ionophore (PTM14C4), anionic dye (LAD-3), and lipophilic organic liquid (TFPDE). Mixing ratio of the ion-sensing components, PTM14C4/LAD-3 (in mol/L) = 0.035/0.035. All sample and flowing solutions contain 0.05 M Tris-HCl, pH 7.

chain of the dodecyl group was attached in the TFPDE molecule so that an optimal lipophilicity of over 10^{10} in log P_{ow} value was attained. An alkyl chain longer than C12 (dodecyl) into this molecule is not favorable because it is no longer liquid at room temperature (20 °C). The typical Li^+ response curve of the sensor based on PTM14C4, LAD-3, and TFPDE as the sensing components is shown as the broken line in Figure 8. Compared with curve c in Figure 7, the sensor sensitivity was increased about 1 order of magnitude, and a lower detection limit (1×10^{-6} M Li^+) was attained. Because of the limiting solubility of LAD-3, this detection limit is the lowest among all prepared Li^+ optodes. On the other hand, the Li^+ selectivity of this sensor is slightly lower than that of the sensor using BEHS. The Li^+ sensor using TFPDE responded to Na^+ ($\log K_{\text{Li,Na}}^{\text{opt}} = -4.3$ by SSM at 10^{-1} M cation concentration), but did not response to all other alkali metal and alkaline earth metal cations except Li^+ and Na^+ .

Sensor Application. To prepare the calibration curve for Li^+ in the serum of manic depressive patients, the typical serum ion concentration was adjusted and used to prepare the test samples of various Li^+ concentrations. The typical response curve for the 10-times-diluted artificial serum samples is shown as the solid line in Figure 8. Through the test calibration solutions contained typical background ions of 10-times dilution of the upper level ion concentration for a normal human serum (15 mM Na^+ , 0.5 mM K^+ , 0.12 mM Ca^{2+} , and 0.08 mM Mg^{2+}),¹⁷ the Li^+ response curve was in

perfect accordance with the response curve for Li^+ without any interfering ions down to 1×10^{-5} M (0.01 mM) Li^+ . Consequently, it was decided that this optical sensor can be used to measure serum samples containing 0.5–1.5 mM Li^+ , which is the typical concentration range in the serum of manic depressive patients.¹ A typical response profile of the sensor for the 10-times-diluted Li^+ -spiked human serum samples is shown in Figure 9. Concerning the reproducibility of the Li^+ sensor, the relative standard deviations for the 10 measurements of the response to the 1:10 dilution serum samples containing 2×10^{-4} and 1.8×10^{-3} M Li^+ were 1.3% and 1.4%, respectively. About 15 min was required for one determination of the serum sample containing 1.8×10^{-3} M Li^+ serum samples diluted 1:10 with the Tris buffer solution.

The response time for the flow-through sensor was about 60–100 s, which was considered to be determined by the diffusion of the sample in the flowing solution as well as the packing state of the sensing beads, which depends on how the flowing solution travels through the packed-beads section in the flow-through optical cell. When the beads are separately dispersed the time for the color change of the sensing beads was very fast (within a few seconds). Therefore, the response time could be improved by modifying the structure of the flow-through cell. The sensitivity of the Li^+ optode was almost constant for at least 7 days (5–7 h of use per day), but lifetime studies of over 1 week have not been done.

CONCLUSIONS

The flow-through-type Li^+ -selective optode based on the ion-pair extraction reaction with the newly synthesized Li^+ -selective neutral ionophore, PTM14C4, and an anionic dye, LAD-3 (see Figure 3), could detect Li^+ in concentrations ranging from 10^{-6} to 10^{-1} M Li^+ at pH 7.0 with 0.05 M Tris-HCl buffer as the flowing solution by measuring the absorbance change at 516 nm. The response characteristics were theoretically explained, and the expected response behavior was applied to prepare the sensitive Li^+ sensor. This optode has an excellent Li^+ selectivity of over 10^4 against all alkali metal and alkaline earth metal cations and could successfully be used to measure millimolar levels of Li^+ in Li^+ -spiked serum samples for manic depressive patients. Though similar cation-selective optodes using the absorbance mode of a color-changeable dye have been reported^{11,18–24} (for recent reviews, see refs 21–24), high sensitivity and selectivity were only realized when a highly specific ion-selective ionophore and a highly sensitive dye adduct were successfully obtained.

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